

Retrograde Regulation in the CNS: Neuron-Specific Interpretations of TGF- β Signaling

Minireview

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Retrograde signals influence neuronal survival, differentiation, synaptogenesis, and plasticity. Several recent papers describe novel roles for the well-studied TGF- β pathway in retrograde synaptic signaling. While each dissects spatial and molecular aspects of TGF- β signaling in a specific synaptic context, together these studies demonstrate that a specific retrograde signal may be interpreted in diverse, neuron-specific ways. Thus, a neuron's intrinsic properties and its other extrinsic signaling inputs determine its cellular and genomic response to TGF- β .

Communication between neighboring cells is a fundamental part of being multicellular. In the nervous system, communication between a presynaptic neuron and its synaptic target is distinctive in that the two cells form very limited areas of contact, at zones widely separated from the presynaptic cell body. Despite this unique organization, it is not unexpected that, like other neighboring cells in metazoa, postsynaptic cells communicate in important and instructive ways with their presynaptic "neighbors." Indeed, neuronal fate, form, and function are substantially determined by a variety of target-derived secreted and membrane bound molecules, generally termed "retrograde signals" (Fitzsimonds and Poo, 1998).

An important addition to the list of established retrograde signaling mechanisms was made when Corey Goodman's and Mike O'Connor's groups identified Wishful Thinking (Wit) as a presynaptic TGF- β receptor that positively influences the growth and strength of the glutamatergic *Drosophila* larval neuromuscular junction (NMJ) (Aberle et al., 2002; Keshishian and Kim, 2004; Marques et al., 2002). In the 2 years since, NMJ functions of nearly all major TGF- β signaling components have been elucidated, thanks in part to genetic and molecular resources created by prior analyses of TGF- β signaling in other cellular contexts (McCabe et al., 2004; Rawson et al., 2003) (Figure 1). Parallel analyses of TGF- β function outside the NMJ have revealed additional neuronal mechanisms for regulating and interpreting TGF- β signals as well as unprecedented functions for TGF- β signaling in vivo (Allan et al., 2003; Zhang et al., 1997; Zheng et al., 2003).

Retrograde TGF- β Signaling in Homeostatic Control of a Motor Synapse

Larval motor synapses grow substantially in size and strength as the innervated larval muscle expands in size

through normal development. This coordination between transmitter release efficacy and size of postsynaptic cell is often cited as an example of homeostasis, although independent but temporally coordinated control of these events during development has not been completely excluded. Synaptic homeostasis is more clearly evident when motor terminals respond to experimental manipulations of postsynaptic muscle. Thus, decreased muscle excitability accomplished by expression of dominant-negative glutamate receptors or CaM kinase inhibitory peptides results in transmitter release being enhanced by a positive scaling factor sufficient to maintain wild-type levels of synaptic strength (Haghighi et al., 2003). These and related observations establish that activity-responsive retrograde signal(s) from muscle acts via presynaptic receptors to regulate transmitter release, likely by modulating genes and molecules that control functional properties of the motor neuron.

A series of recent papers demonstrate that TGF- β comprises at least part of this retrograde signal. At the NMJ (Figure 1), a TGF- β protein (Gbb) secreted by muscle is sensed by a neuronal TGF- β receptor dimer formed by a type II receptor (Wit) and either of two type I receptor subunits (Sax or Tkv) (McCabe et al., 2004; Rawson et al., 2003). In neurons, TGF- β receptor activation results in phosphorylation of the nuclear MAD (R-SMAD) transcription factor and activation of TGF- β -responsive genes by a pathway that requires Medea, a MAD cofactor (co-SMAD). TGF- β positively influences the growth and strength of motor synapses; thus, loss-of-function mutations in any TGF- β signaling component cause a reduced number of synaptic boutons and a parallel reduction of transmitter release. Consistent with a model in which signaling requires retrograde axonal transport, nuclear phospho-MAD staining as well as the consequences of activating TGF- β receptors in neurons are eliminated when dynein, the retrograde microtubule motor, is inhibited (Allan et al., 2003; McCabe et al., 2003).

A direct role for TGF- β in homeostatic signaling is provided by an elegant experiment showing that the compensatory enhancement of transmitter release observed when either glutamate receptors or CaMKII are inhibited in muscle does not occur when TGF- β signaling is blocked in *wit* mutants (Haghighi et al., 2003). Together, the data establish that TGF- β acts as a muscle-derived retrograde signal to positively regulate synaptic growth and neurotransmitter release.

Many questions and issues remain unaddressed. First, while the requirement for TGF- β is unequivocal, the sufficiency of TGF- β for homeostatic signaling remains to be established. All available data are consistent with an alternative model in which TGF- β plays a permissive role to make neurons capable of responding to primary signals regulated by synaptic activity. While TGF- β clearly influences synaptic growth, most forms of homeostatic compensation observed at this motor synapse occur without accompanying changes in bouton number (Table 1). A permissive model for TGF- β function, combined with the involvement of an as yet unidentified instructive signal, would be one way to ra-

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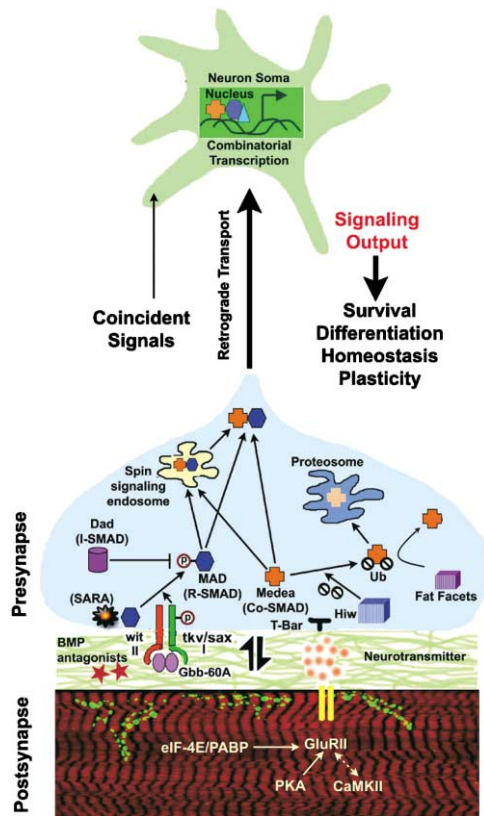


Figure 1. Schematic Representation of Signaling Components of the TGF- β /BMP/Activin Pathway Involved in Neuronal Homeostasis and Plasticity

The conserved signaling module in *Drosophila* includes a target-derived ligand (Gbb-60A) that binds to a complex formed between type I (Tkv or Sax at the periphery, Babo in the CNS) and type II (Wit in the PNS, Wit or Punt in the CNS) BMP receptors. Presumably, upon phosphorylation by the type II receptor, the type I phosphorylates a transcription factor, MAD, possibly causing its release from the membrane anchoring protein SARA, which is not yet investigated in *Drosophila* (Shi and Massague, 2003). Activated MAD now binds a common co-SMAD, Medea, and either via a signaling endosome or through direct retrograde transport reaches the nucleus in the neuronal soma to activate transcription of specific genes. Simultaneous transcriptional activation of a particular combination of genes, determined in part by other intrinsic or extrinsic signals, leads to a precise output (cell survival, differentiation, homeostasis, or plasticity). In the context of homeostatic change, several manipulations at the postsynaptic muscle (such as attenuation of GluRII-dependent response directly or indirectly through PKA, CaMKII, etc.) need robust TGF- β signaling through Gbb, Wit, and Tkv or Sax for retrograde communication. Functional constraints on the quality or duration of this signaling are achieved by inhibitory SMADs (Dad in flies), ubiquitination (by the ubiquitin ligase highwire, *hiw*), and proteasome-dependent degradation of Medea or targeting of the signaling complex for lysosomal degradation (regulated by the *spinster* gene product). A deubiquitinating enzyme encoded by the *fat facets* (*faf*) locus is a positive regulator (DiAntonio et al., 2001). Not studied yet in this paradigm are soluble extracellular matrix bound antagonists of BMP signaling, like Noggin, Chordin, and Follistatin in vertebrates, that might also serve a regulatory role.

tionalize this apparent inconsistency. Thus, further experiments are required to convincingly discriminate between “permissive” and “instructive” models of TGF- β function.

Second, while TGF- β is shown to be required for one form of synaptic homeostasis, its roles, if any, in other homeostatic processes that operate even at this single synapse remain to be established (Table 1). For example, a synapse with fewer presynaptic varicosities, created by manipulating levels of fasciclin II, shows normal synaptic strength due to a compensatory increase in quantal size (Davis and Goodman, 1998). Does the TGF- β pathway that influences presynaptic growth and transmitter release underlie this mechanistically distinct form of synaptic homeostasis?

Finally, while muscle-derived TGF- β is shown to influence presynaptic properties, the underlying mechanisms remain unknown. In the future, it will be productive to explore potential interactions with other signaling pathways that regulate the growth and strength of the motor synapses, e.g., PKA, MAPK, CREB, and AP-1.

Varied Neuronal Interpretations of TGF- β Signaling

While it would be presumptuous to predict that TGF- β signaling will be involved in all forms of synaptic homeostasis, it would be wholly incorrect to postulate synaptic homeostasis as the only neuronal function of the TGF- β pathway. For instance, roles for TGF- β in mammalian neuronal development and/or survival are indicated by observations that mouse SMAD4 mutants show a large increase in the cerebellar Purkinje cell number, mouse TGF- β mutants show greatly increased neuronal apoptosis, and that a TGF- β family member (BMP9) controls cholinergic differentiation of mouse spinal cord and septal neurons (Brionne et al., 2003; Lopez-Coviella et al., 2000; Zhou et al., 2003). Mechanisms that underlie similar diverse roles for TGF- β signaling in neuronal differentiation, remodeling, and plasticity have been outlined by recent elegant studies in invertebrate model organisms.

In peptidergic Tv neurons of the *Drosophila* CNS, expression of the neuropeptide FMRF is initiated subsequent to target cell innervation (Allan et al., 2003; Marques et al., 2003). A beautiful analysis of intrinsic and extrinsic factors that govern the differentiation of Tv neurons shows axon pathfinding to be dependent on expression of the transcription factors Apterous and Squeeze. However, FMRF expression in these cells is induced by target-derived TGF- β (Gbb) acting through a presynaptic TGF- β receptor, likely a Wit/Tkv or Wit/Sax dimer. Thus, in Tv neurons, retrograde signaling by TGF- β underlies not homeostasis but appropriate biochemical differentiation of the peptidergic neuron. Here, TGF- β signaling acts as an extrinsic instructive input to a combinatorial transcription factor code that governs FMRF expression, a conclusion strengthened by the unambiguous demonstration that combining activation of TGF- β signaling with Apterous and Squeeze misexpression triggers ectopic FMRF expression in peptidergic neurons (Allan et al., 2003). Similar instructive roles for TGF- β signaling have been reported in the nematode nervous system (e.g., Nolan et al., 2002).

In mushroom body Kenyon cells, TGF- β signaling plays a role distinct from its function in motor or Tv neurons (Zheng et al., 2003). During metamorphosis, Kenyon cells are remodeled from their larval form in which they innervate both medial and dorsal lobes of the mushroom body to the adult form in which they

Table 1. Homeostasis at the *Drosophila* Larval Neuromuscular Synapse

Experimental Manipulation	Compensatory Response	Growth	TGF- β Required
1. Increasing bouton number	reduced transmitter release	–	unknown
2. Decreasing bouton number	increased quantal size	–	unknown
3. Electrically silencing muscle	increased transmitter release	no	unknown
4. CaMKII inhibition in muscle	increased transmitter release	no	yes
5. GluR inhibition in muscle	increased transmitter release	no	yes
6. PKA activation in muscle	increased transmitter release	ND	unknown
7. Increased postsynaptic eIF4e	increased transmitter release	yes	unknown
8. Increased postsynaptic PABP	increased transmitter release	yes	unknown

In response to various experimental perturbations of motor neuron or muscle, different compensatory responses are observed, generally directed toward maintaining wild-type synaptic strength. Alterations in bouton numbers in 1 and 2 (by manipulation of FasII levels in muscle) lead to unique, compensatory changes—either reduced transmitter release or increased amplitude of individual quantal events. A compensatory increase in evoked transmitter release associated, in some cases, with an increase in the number of release sites per bouton, occurs following perturbations 3–6. Significantly, no changes in synapse growth have been seen in these cases. Perturbations 7 and 8 result not in homeostatic compensation but rather in processes that drive persistent change in synaptic strength. They are included in this table as they also clearly require retrograde signaling between muscle and neuron. Of these documented instances of muscle-neuron communication, two (4 and 5) have been shown to require TGF- β . (References: Davis and Goodman, 1998; 2, Stewart et al., 1996; 3, Paradis et al., 2001; 4 and 5, Haghighi et al., 2003; 6, Davis et al., 1998; 7 and 8, Sigrist et al., 2000).

innervate only the medial lobe. Larval axons retract from both lobes and then regrow into the medial γ lobe by a mechanism that involves regulation by the steroid hormone ecdysone. In this process, TGF- β renders larval neurons responsive to steroids that direct axonal remodeling. Using the MARCM method to visualize individual mutant neurons, Tzumin Lee's group found that loss of TGF- β signaling components, a type I receptor (Babo), type 2 receptors (Wit or Punt), or SMAD2 led to the persistence of the larval Kenyon cell morphology. Remarkably, the requirement for TGF- β signaling may be relieved by heterologous expression of ecdysone receptor, observed to be reduced in mutant cells. Thus, in Kenyon cells, TGF- β /Activin, possibly released by target tissue, plays a permissive function in which it induces expression of a receptor protein that confers responsiveness to primary regulators of axonal remodeling (Zheng et al., 2003).

Potential Mechanisms to Explain the Diversity of TGF- β Responses

Diverse neuronal responses to TGF- β may be explained by one of three classes of mechanism. First, substantial complexity may be inherent in the diversity of ligands, receptors, and TGF- β pathway components. Second, if levels, duration, or waveforms of TGF- β signaling are significant, as indeed they appear to be for calcium and MAP kinase signaling, then, for instance, the TGF- β pathway activated in Tv neurons may differ qualitatively or quantitatively from TGF- β signaling activated in motor neurons in ways not apparent to recent genetic analyses. Third, intrinsic differences between neurons could lead to different interpretations of identical inputs from the TGF- β pathway. We briefly consider these not mutually exclusive possibilities below. The reader is directed to other reviews of TGF- β signaling for a more comprehensive discussion (e.g., Shi and Massague, 2003).

In mammals, a diversity of cell type-specific receptors and nuclear SMADs mediate a qualitatively diverse set of TGF- β signals (Shi and Massague, 2003). This, however, appears to be a limited source of diversity in *Drosophila*, in which MAD and SMAD2 appear to be the only two TGF- β -coupled transcription factors.

The duration and intensity of TGF- β signaling in neu-

rons may be modulated by a variety of other potentially cell type-specific mechanisms, including, most trivially, varied expression levels for TGF- β signaling components (Shi and Massague, 2003). In *Aplysia*, where the first evidence for TGF- β 's role in regulating synaptic plasticity was discovered, increased synthesis of a Tolloid-like metalloprotease (that cleaves and thereby activates extracellular inactive TGF- β) is associated with long-term facilitation (LTF) at the sensorimotor synapse; indeed, exogenous TGF- β appears to be largely sufficient to induce LTF (Zhang et al., 1997). After receptor activation, TGF- β signaling may be prolonged by the transport of receptors to a class of signaling endosome (Shi and Massague, 2003) or limited by targeting them via the endosomal protein Spinster, to degradative, lysosome bound endosomal compartments (Sweeney and Davis, 2002). A parallel degradative mechanism is suggested by the observation that Medea (Co-SMAD) is found associated with Highwire, a presynaptically enriched ubiquitin ligase (McCabe et al., 2004). Instructively, loss-of-function mutations in both *spinster* and *highwire* lead to greatly expanded synapses, a predicted (but only modestly documented) consequence of enhanced TGF- β signaling (Sweeney and Davis, 2002; Wan et al., 2000). Thus, levels or activities of Tolloid or of specific endocytic and ubiquitin-mediated degradative pathways could act to initiate, sculpt, or otherwise modify primary TGF- β signals (Figure 1). Cell type-specific activities of these components or other intersecting signaling pathways could account for the differences in TGF- β signaling in different types of neurons.

But how are TGF- β signals decoded? Although likely, at this time there remains little evidence that information is encoded in subtle qualitative details of TGF- β signaling. However, dramatic differences between, for example, responses of Tv neurons and motor neurons to TGF- β clearly arise from differences in the transcriptional ground states of neurons (Allan et al., 2003). In Tv neurons that, unlike motor neurons, express Apterous and Squeeze, activation of nuclear MAD results in FMRF gene expression. Other classes of neurons that do not express Apterous and Squeeze do not express FMRF in response to MAD activation; however, ectopic expres-

sion of Apterous and Squeeze in these cells can make FMRF expression responsive to TGF- β activation. Thus, the difference in TGF- β responsiveness of these FMRF-silent and Tv peptidergic neurons may be ascribed to differences in the transcriptional ground state, determined substantially by the presence or absence of two identified transcription factors (Allan et al., 2003).

While intrinsic differences between two peptidergic neurons may be relatively simple, a large diversity of transcriptional ground states is likely to exist within the central nervous system. This is indicated by the observation that Apterous, Squeeze, and BMP activation is only sufficient for FMRF expression in the context of peptidergic neurons but not in the majority of other neurons in the CNS (Allan et al., 2003). Assessing the intrinsic diversity of neurons is an issue of considerable significance, not least because it impacts how reasonable it may be to generalize, to the entire nervous system, conclusions drawn from studies in one or a small number of cell types. Thus, as we have tried to outline in this minireview, studying the diversity of neuronal responses to TGF- β and the underlying mechanisms may not only provide insight into basic processes like synapse homeostasis but may also provide a window to an even more fundamental question in neuroscience.

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